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SHORTER ARTICLES AND DISCUSSION

THE INFLUENCE OF CAVE CONDITIONS UPON PIGMENT DEVELOPMENT IN LARVÆ OF AMBLYSTOMA TIGRINUM

ONE of the possible methods of approach to the problem of the origin of the modifications of cave animals is by experiments in which outside forms are kept under conditions normally encountered by animals living in caves. In following up this method of approach, it seemed best to select forms as plastic as possible (if perchance there are forms which are plastic with reference to modifications by cave conditions). Typical cave animals, *i. e.,* species highly adapted for cave life, perhaps without exception belong to families and genera having many members showing an inclination toward cave habitation either by actually frequenting or living in caves or by inhabiting similar dark and retired situations elsewhere; so that it might seem that these groups possess a certain plasticity toward modifications by cave conditions. Especially might this plasticity be expected in the particular species which already show a tendency to live under conditions resembling those of caves.

Young animals may be expected to be more responsive to changed environment than adults, and since many of the urodeles live in caves and similar situations, and their eggs and young can be obtained in numbers and reared with comparative ease, amphibian larvæ were selected for some of the experiments.

Newly laid eggs of *Amblystoma tigrinum* were collected (in some cases were laid in jars in the laboratory where some of the adults were confined) from March 30 to April 4, 1911. The eggs, in small lots, were placed in a number of 6-inch battery jars, and the jars divided into two lots, Series *A* and Series *B*. Series *A* was placed in the artificial cave while Series *B* was kept on a laboratory table adjacent to and directly in front of a west window. Otherwise the two series were, as far as possible, subjected to like conditions. Unfortunately it was not possible to keep Series *B* at as low or as uniform a temperature as Series *A*. Development was somewhat slower on the average with Series *A* than with Series *B,* and both of these developed slightly less rapidly than a third series, Series *C,* observed in the pool where the eggs were laid and the larvæ allowed to develop under natural conditions, but as I shall attempt to show later neither temperature differences nor different rates of development materially influenced the amount of pigment developed.

The animals were fed on alternate days with an abundance of daphnids and cyclops, and later with small bits of tender beef. The amount of pigment developed was judged by making accurate determinations of the color of two definite regions of the skin of each individual. For the body color, determinations were made for a region to one side of the median line and about midway between the pectoral and pelvic limbs. This is the most heavily pigmented region of the larva. For the head color the region immediately anterior to a line from one eye to the other was used. This is usually the least pigmented portion of the animal. The colors were obtained by means of the Milton Bradley color tops and the records made in percentages of black, white, orange and yellow, which when blended most nearly matched the color of the skin of the animal. The orange used in these tops most nearly resembles the No. 101 of Klincksieck et Valette's "Code des couleurs" and the yellow is intermediate between Nos. 206 and 211. All the color records under consideration for Series *A* and *B* were made within two weeks and under uniform conditions so that the results ought to be strictly comparable. The records for the somewhat more rapidly developing Series *C* were made about three weeks earlier. The results tabulated include color records for all the surviving individuals of Series *A* and *B* and sixteen individuals selected at random to constitute Series *C*.

The average body color of the Series *A*, numbering 22 individuals, reared in the cave, contained 49.7 per cent. of black, 16.9 per cent. white, 9.3 per cent. orange and 24.1 per cent. of yellow; or 49.7 per cent. black and 50.3 per cent. non-black. The extreme range in degree of pigmentation was from 32 black and 68 non-black to, in the case of one individual decidedly darker than its fellows, 70.5 black to 29.5 non-black. The average head color was 38 per cent. black and 62 per cent. non-black (26.7 white, 7.0 orange and 28.3 yellow) with 27 per cent. and 51 per cent. as the extremes in amounts of black.

On the average, Series *B* (7 individuals), reared in laboratory light, had a body color of 86.1 per cent, black and 13.9 per cent. non-black (4.7 white, 2.4 orange and 6.8 yellow), and a head color of 76.1 per cent. black and 23.9 per cent. non-black (8.4 white, 4.0 orange and 11.5 yellow). The range for the body color was from 82 to 90.5 black and for the head color from 62.5 to 90 black, ranges distinct from and not at all overlapping those for Series *A* reared in darkness.

Series *C* (16 individuals), living in the outdoor pool under natural conditions, had an average body color of 86.6 black and 13.4 per cent. non-black (4.5 white, 3.1 orange and 5.8 yellow), the extremes ranging from 77.5 to 93 per cent. black. The head color averaged 78.3 black and 21.7 non-black (7.7 white, 4.2 orange and 9.8 yellow) with 61 per cent. and 90 per cent. the extremes in amount of black. The data for all three series are shown together in the adjoining table.

	Body					Head				
	Black	White	Orange	Yellow	Total Non-Black	Black	White	Orange	Yellow	Total Non-Black
Series *A* (cave):										
Extremes:										
Darkest	70.5				29.5	51.0				49.0
Lightest	32.0				68.0	27.0				73.0
Averages	49.7	16.9	9.3	24.1	50.3	38.0	26.7	7.0	28.3	62.0
Series *B* (laboratory):										
Extremes:										
Darkest	90.5				9.5	90.0				10.0
Lightest	82.0				18.0	62.5				37.5
Averages	86.1	4.7	2.4	6.8	13.9	76.1	8.4	4.0	11.5	23.9
Series *C* (pool):										
Extremes:										
Darkest	93.0				7.0	90.0				10.0
Lightest	77.5				22.5	61.0				39.0
Averages	86.6	4.5	3.1	5.8	13.4	78.3	7.7	4.2	9.8	21.7

It will be noted that the series reared in laboratory light (Series *B*) and the one developing under natural conditions in the outdoor pool (Series *C*) agree very closely in the amount of pigment development, the average percentages of black in the body color determinations being 86.1 and 86.6, and for the head color 76.1 and 78.3 respectively, while the extremes for the two series differ but slightly. However, the most significant fact is that these series differ so sharply from Series *A* reared in the dark and having an average body color of only 49.7 per cent. black and an average head color of .38 black. On the average those reared in darkness have about four times as much non-black in the body color as those reared in light, while the darkest individual of the cave series, really aberrant for that series because it is so dark with 70.5 per cent. black in the body color and 51 per cent. in the head color, has decidedly less pigment than the lightest individual of either Series *B* or Series *C*, with 77.5 per cent. black in the body color and 61 black in the head color.

The percentage differences in color between the series reared in darkness and the two series reared in light do not adequately represent the color differences as perceived by the eye. The average person probably would not hesitate to call the lighter individuals of Series *A* "white," and on the other hand would probably class the various individuals of Series *B* and *C* as "dark gray" or "coal black."

There appear to be no reasons for thinking that the marked differences in pigment development were due to differences in nutrition or differences in temperature. Series *B* so closely resembled Series *C* in amount of pigment development that it is evident the differences in temperature and food did not greatly influence their pigmentation, yet Series *B* was reared in a west room subject to great fluctuations in temperature because of the extreme exposure to the summer sun during the afternoon, and the temperature of this series on the average was probably higher than in the outdoor pool in which Series *C* developed, while the food was exclusively daphnids, copepods and bits of beef. On the other hand Series *A* was maintained at a quite uniform temperature consistently lower than either of the other series. Fischel ('96) and Flemming ('97) found that with certain salamander larvæ (the former at least used larvæ of *Salamandra maculata*) temperature differences influenced the amount of pigment development, the individuals reared at from 4° to 7° C. being much darker than individuals reared at from 15° to 20° C., indicating that for *Salamandra maculata* the lower temperature favors greater pigment development. Inasmuch as my lightest series, Series *A*, was reared at the lowest temperature of any and yet was markedly lighter than the others, and that Series *B* and *C* did not differ widely in amount of pigment development although reared under somewhat different temperature conditions renders it improbable that temperature influences produced the differences in pigmentation observed.

Series *A* and *B* were fed in every way alike and so far as could be judged took about the same quantities of food. While as a whole Series *A* developed less rapidly than Series *B*, part of Series *A* developed more quickly than some of Series *B* yet each individual possessed an amount of pigment similar to the others of its series. Hence the evidence strongly points to the conclusion that the differences in amount of pigmentation were not due to differences in temperature, food or rate of development but solely or in much the greater part to the absence or presence of light.

The difference in pigmentation between Series *A* on the one hand and Series *B* and *C* on the other is fully as great as the difference in pigmentation between certain species that live habitually in caves and others which do not. To this point the case looks most significant. With the approach of transformation, however, the amount of pigment rapidly increases, particularly on the lighter regions of the larvæ, and this increase is more pronounced with those reared in darkness than with the others. Nevertheless there still remains a marked difference in pigmentation between the transformed individuals of Series *A* reared under cave conditions and Series *B* reared in the light.

Color records of a few recently transformed salamanders were taken before the spots so characteristic of the adult were definitely formed. An attempt was made to get careful records of the general blended color effect independent of the mottling which is quite pronounced at this stage, during which the pigment is being segregated to form the definite color pattern of the adult. The average body color of the transformed salamanders of Series *A* was 70.2 per cent. black and 29.8 per cent. non-black, and the average head color 68.2 black and 31.8 non-black. These averages for Series *B* are: Body color, 89.7 black and 10.3 non-black; head color, 87.7 black and 12.3 non-black. No transformed individuals of Series *C* were obtained. Unfortunately records of transformed individuals of Series *A* and *B* were made of only 2 and 5 individuals, so these are scarcely more than sample records, but they are probably fairly representative of their respective series, and they clearly indicate that after, as well as previous to, transformation a distinct difference in pigmentation persists between the salamanders reared and allowed to transform in the cave and those developing and transforming in the light. A. M. BANTA

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